Consensus statements from the Second International Lung Cancer Molecular Biomarkers Workshop: A European strategy for developing lung cancer molecular diagnostics in high risk populations

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Abstract. The Second Molecular Biomarkers Workshop was held at the Roy Castle International Centre for Lung Cancer Research in Liverpool, in June 2001 and it brought together experts in the clinical, epidemiological and molecular-pathology of lung cancer from Europe and the USA, to address issues surrounding the development of a European strategy for early lung cancer detection. The 2001 Workshop Breakout Groups concentrated on the current challenges in the early detection of lung cancer which need to be addressed in the light of the recent surge in interest in many countries for mounting new clinical trials to evaluate the utility of Spiral CT in early lung cancer detection. If population-based trials of CT screening are mounted it will also be a favorable clinical environment in which to evaluate efficiently recent advances in molecular screening and genotyping. The Workshop focused specifically on: a) clinical and molecular biomarkers, b) sputum as an early detection and diagnostic tool, c) validation of molecular markers prior to their use in early detection trials and d) ethical issues that have to be considered in early lung cancer detection trials. A distillation of the Workshop discussions is given in this article.

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Contents

4. Molecular biomarkers should be validated before they are used in early detection trials
5. Ethical issues that have to be considered in early lung cancer detection trials

1. Introduction

The Second Molecular Biomarkers Workshop held in Liverpool in June 2001 brought together experts in the clinical, epidemiological and molecular-pathology of lung cancer from Europe and the USA, to address issues surrounding the development of a European strategy for early lung cancer detection. The deliberations of the first Workshop were recently published in Lung Cancer (1).

The 2001 Workshop Breakout Groups concentrated on the current challenges in the early detection of lung cancer which need to be addressed in the light of the recent surge in interest in many countries for mounting new clinical trials to evaluate the utility of Spiral CT in early lung cancer detection. If population-based trials of CT screening are mounted it may also be a favorable clinical environment in which to evaluate efficiently recent advances in molecular screening and genotyping. A distillation of the Workshop discussions is given in this article. This article is written to frame topical questions and provide current thought on these issues in this fast moving area.

2. Population-based early lung cancer detection: clinical and molecular biomarkers

The data suggesting that Spiral CT represents a major step forward in lung cancer detection continue to build, as does the support for mounting new early lung cancer detection validation trials. Innovations in other related areas include the use of computer-assisted diagnosis tools as well as less invasive surgical techniques for the small-volume primary lung cancers that may be detected. While it is encouraging to have these rapidly developing new tools for early lung cancer management, the design of a large, definitive randomized trial remains difficult. Workshop investigators concurred that having very large numbers of trial participants would help to ensure that statistically meaningful conclusions would emerge but might be difficult to achieve in any one trial. Since no single trial design emerged as being clearly preferred, the fallback position was to harmonize on as many elements of the trials as possible. This would permit combined analysis of all of the individual trial results in a fashion that may provide some definitive answers. An example of this type of deliberation includes study parameters such as the criteria for inclusion into the study. Some groups favoured older smokers with greater tobacco consumption habits while other investigators were concerned that the results of this type of study would not generalize to younger smokers where the possibility of improving premature loss of life would be greater.

The selection of study populations for the validation trial generated an enormous amount of discussion. However, regardless of the trial designs, it was considered imperative that standardized provisions were adopted. This suggestion, led investigators at the Workshop to consider pooling the current Spiral CT case-control and cohort studies for subgroup analysis. This is especially relevant when it is considered that, internationally, there are over 12 European/US and Japanese Spiral CT trials currently running or in the planning stages. There is a recognized trade off between generalizability vs. efficiency when designing early detection trials, however, the value of integrating the developments in Spiral CT imaging technologies with the emerging field of molecular biomarkers cannot be overstated. It was also agreed that the design of these studies should take on board the major cause of lung cancer, smoking, and thus, where possible, include former smokers as well as current smokers in their trial designs. Whenever possible, biospecimens should be collected from participants in these trials at baseline and at each recall visit.

Biospecimens that should be considered in an early lung cancer detection trial. a) Serum/plasma; b) Frozen whole blood, Guthrie card, RNA; c) Viable lymphocytes; d) Sputum, bronchial lavage, brushings, biopsy specimens; e) Oral rinse/brushings; f) Urine; g) Tissue, including precursor lesions, tumour and adjacent normal remain the highest priority. The development of such an archive associated with early detection trials will be of immeasurable value in facilitating related research in the future.

3. Sputum as an early detection and diagnostic tool

In early lung cancer detection studies, one question that is repeatedly asked is whether sputum may be used as a reliable diagnostic tool and what are the problems. The consensus of this Workshop was that sputum is a promising specimen source for the early recognition of cancer present in the airways. In brief, early lung cancer detection methods should be based on specimens that may be easily obtained such as sputum and plasma. While plasma may indicate the presence of an already established tumour, sputum may permit detection of much earlier lesion. Furthermore, sputum locates the cancer to the airways and is therefore organ specific. Sputum provides DNA, RNA, protein and morphology for detection of early cancer, all of which may be assessed provided it is correctly collected and archived. a) DNA: risk markers, allelic imbalance, methylation status and specific mutations (e.g., K-*ras*); b) RNA: expression arrays; c) Protein: immunohistochemical (or phenotypic) markers (e.g., hnRNP); d) Morphology: automated cytometry.

Although none of the currently available biomarkers have been validated preliminary data are exciting and hold the promise for detection of biomarkers in sputum to be complementary to the Spiral CT detection for early lung cancer. All histological types of lung cancer are detectable in sputum with modern methods (DNA and automated image cytometry). The collection of sputum during Spiral CT screening will offer an unprecedented opportunity to evaluate the role of sputum markers especially in bolstering the detection of centrally arising squamous cancers that may not be as readily detected with CT as the peripheral adenocarcinomas. The group agreed about the importance of refining and standardizing the collection and archiving of specimens, so that the sensitivity and specificity of individual sputum tests can be reliably and reproducibly determined.

Further comparative studies are needed to determine the optimal specimen collection methods for particular assays, for the present, with new lung cancer screening trials, the Breakout Group recommends a minimal sputum collection protocol as follows: 15-min saline induction has been most consistently associated with adequate sample generation, but 3-day morning collection can provide satisfactory specimens. If possible, immediate processing of sputum with dithiothreitol (DTT), to remove mucus, eliminates the confounding effect of precipitated mucous strands aggregating the cellular fraction of the specimen. Room temperature preservation in Saccomanno's preservative (2% polyethylene glycol in 50% ethanol) will satisfactorily preserve sputum for most of the tests above. This minimal protocol will not preserve RNA, and may result in DNA cross-contamination (during blending to shear the mucous strands). If aliquots (e.g., for DNA testing) are required, addition of DTT followed by vortexing will maintain the integrity of the DNA across aliquots. Several strategies will preserve RNA: immediate freezing in liquid nitrogen; addition of RNA-later (Ambion); addition of TRIzol®; immediate addition of DTT, wash, EDTA, wash and viable freeze (DMSO/FBS suspension) in liquid nitrogen.

Are enrichment techniques feasible/reliable for sputum induction in large population based studies? Sputum induction is enhanced by chest wall oscillation. Pilot studies have suggested that wearing an oscillating vest during the 15-min saline inhalation is well tolerated and results in improved cell production and rates of satisfactory specimens by standard cytological criteria. However, this may not be feasible in most sputum collection series. New induction agents, alternative to saline, may help to improve cell yield and rate of satisfactory specimens. Large scale evaluations will be required before definitive conclusions can be made about the optimal technique to ensure the most useful sputum specimen.

The investigators in the Breakout Group were of the opinion that magnetic bead enrichment has not worked well in mucus containing suspensions. Enrichment may or may not be essential in the context of helical CT-detected lesions.

The role of free DNA in early lung cancer detection is not yet understood. Thus cell enrichment in this regard may be counter-productive.

Is automation a feasible option for cytomorphological lung cancer detection and what are today's problems in resolving this? Preliminary studies of automated systems are promising. The FDA (USA) has approved automated cytometry for cervical cancer. Opportunities now exist for automated cytometry of sputum. The participation in existing programs of validation of markers such as the Early Detection Research Network (EDRN) was encouraged. Centers that collect specimens and laboratories that develop markers may participate in this validation program through Associate Membership. Applications are available at http://edrn.nci.nih.gov.

The diagnosis of lung cancer requires a multidisciplinary approach. However, classical cytology remains the gold standard to assess the presence of malignant cells and an excellent classical cytology assessment is still necessary during validation of sputum molecular diagnostics.

4. Molecular biomarkers should be validated before they are used in early detection trials

The framework for this aspect of the discussion is the identification and development of assays for biomarkers that would provide a mechanism for the detection of early lung cancer. Since 20% of the world's population is at-risk for lung cancer based on tobacco use and we recognize that the scope of the problem requires practical and economical solutions. How do we set up biomarker validation methods?

Firstly, we need to define the term, biomarker; molecular biomarkers reflect genetic alterations and/or molecular pathway changes, which can be detected and are of clinical relevance for prognosis, staging, grading, early detection, or treatment decisions. These markers may include changes that are present in precursor or cancer cells or those which are somewhat differently expressed in altered cells. Our specific focus is on those biomarkers that would prove useful in early detection. Targets for biomarker assays include tissue and or body fluids.

The validation of biomarkers should be considered at various levels: a biomarker used in early detection of lung cancer must have certain characteristics. First, the biomarker must be reliable, reproducible and robust in the laboratory setting. Standardized methods are required to assess performance as well as the inclusion of gold standard controls for each biomarker. Mechanisms for certifying laboratory performance (accreditation) are needed. Along with assay performance, a biomarker must be shown to be clinically relevant in determining the presence of lung cancer, and especially including pre-invasive lesions. The biomarker must have the same characteristics when moved to commercial scale and this is recognized as a significant hurdle, which will require commercial cooperation. Finally, a biomarker should be evaluated in an appropriately designed and adequately powered study with lung cancer mortality as a final endpoint.

From a practical aspect, biomarkers are best considered within genetic or molecular systems since different types of biomarkers will utilize the same materials (DNA, RNA, protein). Within genetic systems, individual genes and/or pathways may be examined.

Classes of markers - possibilities and opportunities. a) DNA methylation: epigenetic markers; b) Functional markers in lymphocytes, breakpoints at critical markers i.e., DNA repair: COMET assay; c) Microsatellites/LOH/methylation assay in circulating tumour DNA; d) Cytogenetic markers, especially identification of breakpoints; e) Proteomics: general, and specific growth factors; f) Expression markers: in circulating tumour cells compared to tumour tissue; g) Circulating tumour cells: are these detectable as early markers or as reflections of tumour burden? h) Genetic polymorphisms, recommend focus on pathways: i.e., phase 1, 2, DNA repair genes; i) DNA adducts or other markers: in lymphocytes or haemoglobin - a) - smoking exposure assays: i.e., cotinine levels and genetic polymorphisms which may be related to cotinine levels and/or separately to nicotine addiction e.g., DRDZ Taq A1/A2.

The process of biomarker validation is a complex process encompassing several interrelated stages. *A priori* considerations include assays that can be performed on easily collected, processed and stored samples that are economically

sustainable. Issues of assay performance characterization (as discussed above) will need to be addressed. These issues include the biological relevance of the molecular target of the assay as well as characteristics of the assay itself. A strong sense from the group was to facilitate the final validation of particular markers in the setting of specimen archives derived from ongoing large and well-designed trials. This cooperation would allow validation to be more easily achieved and at a much more approachable cost, if longitudinal studies are initiated, such as the Liverpool Lung Project (2), even before tested biomarkers are available. Validation should proceed in stepwise fashion so that sample resources are preserved.

The Group recognises that automation is an essential initial investment in the development of any biomarker assay to be useful in population-based application, especially given the scope of lung cancer early detection. The costs of this initial investment are likely to be rapidly recouped in savings during actual use of such assays. Biomarker assays that can be readily automated are the most likely to be successful in large-scale settings.

For a number of reasons, existing and planned Spiral CT studies are ideal opportunities for evaluating the value of biomarkers compared to imaging screening. This evaluation allows adaptive complementary roles for such methods to be defined.

Is it possible for the scientific community to prioritise biomarkers? One of the difficulties in prioritisation is the rapid development of our understanding of lung cancer biology and diverse technologies. Existing mechanisms for prioritisation include funding mechanisms and external grant review as well as the focus of industrial sponsorship. Since progress in this area has been so vexing, these means of prioritisation may not be adequate. One way to address this deficiency is to form international consortia of individuals with expertise in molecular diagnostics. The consensus from this Workshop would be to include representatives from granting agencies and industry in addition to research scientists. Initial existing examples of efforts in this regard include the Lung Cancer Specialized Program of Research Excellence (SPORE) and Early Detection Research Network (EDRN) in the US and the EU Early Lung Cancer Detection Group (EUELCDG). Although additional resources are justified in light of the significant public health benefit that could emerge through this line of research, mechanisms for encouraging the participation of relevant groups of researchers in this dialogue should also be developed.

A final concern for prioritisation is the acquisition of samples associated with defined outcomes. We recognize that tremendous time and costs are involved in generating a useful archival resource. A dialogue between investigators and funding agencies will be necessary for optimal collection, archiving and utilization.

5. Ethical issues that have to be considered in early lung cancer detection trials

Ethical points that should be addressed in patient information and consent forms. a) Broad consent that takes into account the certainty of the technological evolution. b) Emphasis on RESEARCH nature of findings (avoids insurance issues). c) Make distinction regarding the type of biomarker studies needed for screening, diagnosis, post-treatment monitoring vs. those for single gene disorders. d) Classify molecular genetic and other biomarker testing as RESEARCH rather than CLINICAL. e) Lung cancer screening studies should develop consensus informed consent instruments that review salient information in a form that an average person can understand. f) Develop tailored consent documents that outline the cost and benefits of participating in clinical trials with the intention of developing new clinical tools for early lung cancer detection. g) Include smoking cessation and maintaining abstinence (in recent ex-smokers) options in early lung cancer detection trials where possible to underscore the proven public health benefit of smoking cessation and avoiding relapse and the likelihood that there are biomarkers, which may influence nicotine addiction and smoking cessation.

Challenges with public dissemination of population-based approaches to lung cancer care. There is the potential for insurance companies to change the cost of their products based on information gleaned from particular detection tests. As there are no validated clinical tools to support objectively such risk stratification for now, this would not be justified. Further, insurance companies should not be permitted to use genetic test data for underwriting purposes until: a) their utility has been scientifically established; and b) their relevance for insurance practice has been fully evaluated. The criteria used by insurance companies should be appreciated when adopting these stances. Clearly in different national settings different business models exist as to how the insurance industry determines the cost of their products. A summary of the major models is provided below.

Models of insurance. a) Mutuality: insurance that pools risk and is 'underwritten' on actuarial grounds. i.e., premiums based on assessed risk and sum (or level of service) provided e.g., motor insurance. b) Solidarity: insurance that is not underwritten. All subjects treated the same. Premiums based only on sum (or level of service provided) e.g., national insurance. c) Taxation: services provided to all (but may be subject to means test). Contributions based on ability to pay e.g., NHS in UK.

Mutuality and solidarity. If it is accepted that no humane society will abandon those in need of chronic care, provision of adequate revenues to support such care must be implemented through a solidarity rather than mutuality model. As genomics (and other medical advances) will eventually allow predictions of individual morbidity and mortality to become more precise, the case for insurance based medical care may be undermined. From a UK perspective, the only viable and humane solution remains a comprehensive universal health service.

Public concerns concerning genotyping. From an enormous and charged literature, it is evident that the public is very concerned about privacy and gene screening issues. This largely is a separate issue from early detection of a predictably lethal somatic cancer. Unresolved issues such as 'whose genes are they anyway?' which have charged the discussion in the lay press have to be carefully catalogued in regard to their relevance to the current discussion. The question in the

public's mind is who has access to their genetic information. In parallel, careful procedures must be developed regarding protection of early cancer detection information although in a comprehensive health care system such as in the UK, there may be less formidable challenges to overcome than in countries with fragmented insurance systems.

Communicating to the medical professional and the public about this research. Investigators researching early lung cancer detection should be encouraged to work with the public especially through established lung cancer advocacy groups. There is an emerging challenge to relate to the public about research progress in the area of the detection and management of early lung cancer with the potential to convert basic advances to therapeutic and public health benefits. In the UK, with a 5-year mortality rate for lung cancer that is amongst the highest in the developed world, there is certainly a pressing need for greater public support for lung cancer research. The Public needs to be educated how this type of early lung cancer detection research may translate into significant improvements in lung cancer outcomes in a parallel fashion to the promising trends that are emerging with early detection efforts with breast and colon cancer. Based on a series of promising new developments, innovative approaches are required to recast this issue in the public's eye.

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References

1. Field JK, Brambilla C, Hirsch FR, Hittelman W, Hogan M, Marshall D, Mulshine JL, Rabbitts P, Sutedja T, Watson A and Weiss S: Molecular Biomarkers Workshop: A European strategy for developing lung cancer molecular diagnostics in high risk populations. Roy Castle International Centre for Lung Cancer Research, Liverpool, UK, October 1999. Lung Cancer 31/2-3, pp339-345, 2001.
2. Field J and Youngson J: The Liverpool Lung Project: a molecular-epidemiological study into early lung cancer detection. Eur Respir J (In press).